ENVIROLOGIX QUICKTOX KIT FOR QUICKSCAN DON3 QUANTITATIVE DON TEST KIT

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GENERAL INFORMATION

The Envirologix QuickTox Kit for QuickScanDON3 test kit uses lateral flow test strip technology that provides quantitative DON (vomitoxin) test results.

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMB) of the Field Management Division for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMB by phone at 816-891-8403 or email at Patrick.J.McCluskey@udsa.gov.

Approved Test Kit Information	
Test Kit Vendor	EnviroLogix Inc. 1- 207-797-0300
Test Kit Name	QuickTox Kit for QuickScan DON3
Product Number	AQ 254 BG
Effective Date of Instructions	12/11/2014
Conformance Range	0.5 – 5.0 ppm
Number of analyses to cover Conformance Range	1
Type of Service	Quantitative
Supplemental Analysis	No
Extraction Method	For corn gluten meal, malted barley, DDGS and corn gluten feed: Shake 50 gram sample with 200 ml of distilled or deionized water by hand for 30 seconds. For corn, barley, wheat, wheat bran, wheat middlings, rough rice, soybean meal corn flour and corn germ: Shake 50 gram sample with 250 ml of distilled or deionized water by hand for 30 seconds. For wheat gluten: Blend 50 gram sample with 250 mL of distilled or deionized water using a blender at highest speed for 1 minute. For milled rice and sorghum: Shake 50 gram sample with 300 ml of distilled or deionized water by hand for 30 seconds. For wheat flour and oats: Shake 50 gram sample with 300 ml of distilled or deionized water by hand for 1 minute.
Approved Commodities	Corn, wheat, barley, corn gluten meal, corn flour, corn germ, corn gluten feed, distillers dried grains/with solubles (DDGS), malted barley, milled rice, oats, rough rice, sorghum, soybean meal, wheat bran, wheat flour,

	wheat gluten, and wheat middlings.
Test kit format:	Lateral flow strip.
Detection method:	EnviroLogix QuickScan System.

EXTRACTION PROCEDURES

Different commodities require different extraction ratios and time. Choose your commodity and follow the instructions for extraction listed below:

Note: Allow al testing materials to equilibrate to room temperature before use.

a. <u>Extraction procedure for corn gluten meal, malted barley, DDGS and corn gluten feed</u>

1. Transfer 50.0 ± 0.2 grams ground sample to an extraction container.
2. Add 200 ml of distilled or deionized water and seal the container. Ensure the sample is completely wetted.
3. Vigorously shake the container by hand for 30 seconds.
4. Filter the sample through an approved coffee filter (EnviroLogix ACC 083, Part No. 11434) into a clean collection vessel. This is the filtered (clarified) sample extract for testing.

5. Discard filter paper to gain access to the filtered extract.
6. Proceed to **Test Procedures**.

b. <u>Extraction procedure for corn, barley, wheat, wheat bran, wheat middlings, rough rice, soybean meal, corn flour and corn germ</u>

1. Transfer 50.0 ± 0.2 grams ground sample to an extraction container.
2. Add 250 ml of distilled or deionized water and seal the container. Ensure the sample is completely wetted.
3. Vigorously shake the container by hand for 30 seconds.

4. Filter the sample through an approved coffee filter (EnviroLogix Part No. 11434) into a clean collection vessel. This is the filtered (clarified) sample extract for testing.

5. Proceed to **Test Procedures**.

c. Extraction procedure for wheat gluten

1. Add 250 ml of distilled or deionized water to a mechanical blender jar.

2. Transfer 50.0 ± 0.2 grams ground sample to the jar and seal the jar.

3. Blend at highest speed for 1 minute.

4. Immediately transfer a portion of the extract to a micro centrifuge tube

5. Centrifuge at 2000 x g (not rpm) for 1 minute.

6. The clarified top layer will be used for testing. Proceed to Test Procedures.

d. **Extraction procedure for milled rice and sorghum**

1. Transfer 50.0 ± 0.2 grams ground sample to an extraction container.

2. Add 300 ml of distilled or deionized water and seal the container. Ensure the sample is completely wetted.

3. Vigorously shake the container by hand for 30 seconds.

4. Filter the sample through an approved coffee filter (EnviroLogix Part No. 11434) into a clean collection vessel. This is the filtered (clarified) sample extract for testing.

5. Proceed to **Test Procedures.**

e. **Extraction procedure for wheat flour and oats**

1. Transfer 50.0 ± 0.2 grams ground sample to an extraction container.

2. Add 300 ml of distilled or deionized water and seal the container. Ensure the sample is completely wetted.

3. Vigorously shake the container by hand for 1 minute.

4. Filter the sample through an approved coffee filter (EnviroLogix Part No. 11434) into a clean collection vessel. This is the filtered (clarified) sample extract for testing.

5. Proceed to **Test Procedures**.

TEST PROCEDURES

1. Sample Preparation for 0.5 to 5.0 ppm quantitation range.

   a. Transfer 800 microliters (µL) of DB6 buffer solution into a reaction vial. (Take care not to contaminate DB6 buffer-use a new tip for each test and keep covered when not in use).

   b. Using another **new** pipet tip, add 200 µL of the filtered extract (centrifuged extract only for wheat gluten) into the reaction vial and mix by swirling with the pipet tips and then pipetting up and down 5 times. This is the **diluted sample extract**.

   c. Transfer 200 µL of diluted sample extract into a separate reaction vial.

   b. Place a test strip into the reaction vial containing the diluted sample extract. The arrow tape end of the strip should point into the reaction vial. Develop the strip for **three (3)** minutes.

   c. Immediately, after completion of 3 minutes development, cut off and discard the bottom of the strip covered by the arrow tape, and insert test strip into the QuickScan reader for quantitation.

<table>
<tr>
<td rowspan="2">  </td>
<td>The intensity of the Control Line and Test Line will vary depending on DON levels

←Control Line→
← Test Line →
← Cut Here →</td>
<td>  </td>
<td rowspan="2">Development of a Control Line within 3 minutes indicates that the strip has functioned properly.

Any strip that does not develop a Control Line should be discarded.

A second preparation of the extract (using a fresh buffer dilution) should be made and tested using another test strip.</td>
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USING THE QUICKSCAN SYSTEM

Detailed instructions for use of the QuickScan System are supplied with each unit, and can be found at www.envirologix.com/quickscan.

1. Place the test strip face down in the carrier with the barcoded end closet to the handle.

Place strip in QuickScan carrier

2. Insert the carrier into the reader and the strips are read by touching clicking on the "Read Test" area of the screen. Results are recorded in an electronic worksheet. Allowing the user to report and track data easily.

3. Record results. Results are reported in the conformance range 0.5 to 5.0 ppm. Results above 5.0 ppm are reported > 5 ppm.

SUPPLEMENTAL ANALYSIS

Supplemental analysis is a procedure followed when a result is observed above the upper limit of the concentration range used in GIPSA's test kit performance evaluation. The range for performance evaluation of quantitative deoxynivalenol test kits is 0.5 – 5.0 ppm. Therefore, supplemental analysis would be performed for a result above 5.0 ppm. **There are no approved Supplemental Analysis procedures to report results above 5.0 ppm for this test kit.**

REPORTING AND CERTIFYING TEST RESULTS

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-891-8403 or Patrick.J.McCluskey@udsa.gov).

EQUIPMENT AND SUPPLIES

1. Materials Supplied in Test Kits:

    a. 50 QuickTox strips packed in a moisture-resistant container

    b. 100 Reaction vials

    c. 100 pipette tips

    d. DB6 Extraction Buffer)

2. Materials Required but not Provided:

    a. Distilled or deionized water

    b. QuickScan System. (ACC131), part numbers 10050 + 10198

    c. Timer

    d. Pipettes and tips to deliver 200 µL and 800 µL.

    e. Sample cups with lids. (ACC 012-50) or other extraction vessels (sealable bags)

    f. Approved Coffee filters ACC 083, Part number 11434

    g. Micro centrifuge ACC 64E Part number 11204

    h. centrifugation set-disposables for 50 tests ACC 010 Part number 11214

    i. Osterizer Blender, Model 4094 or equivalent.

STORAGE CONDITIONS AND PRECAUTIONS

1. Storage Conditions:

    a. Test kits should be stored refrigerated between 2 to 8 °C.

    b. Prolonged exposure to high temperatures may adversely affect test results.

    c. Do not open the desiccated canister until ready to use the strips.

2. Precautions:

a. Develop the test for 3 minutes and reading the test strip promptly is required for accurate results.

b. Do not use the test kits beyond the noted expiration date.

c. Protect all components from hot or cold temperatures, when not in use. Do not leave in direct sunlight or in a vehicle.

d. Follow the GIPSA issued procedures to run the test. Deviation from this protocol may invalidate test results reported using the test kit. Proper mixing, along with accurate pipetting are essential for accurate results.

e. Testing Notes:

Take care not to contaminate Buffer solution--use a new pipette tip for each test, and keep covered when not in use. Samples that are not thoroughly mixed and/or accurately pipetted will adversely affect test results. **Avoid foam and particulates during pipetting and ensure that the pipette tip does not become clogged with particulates.** After diluting the sample, the final testing volume in the second reaction vial should be 200 µL. Do not reuse diluted samples.

REVISION HISTORY

Revision 0 (12/11/2014)